Male sex-comb teeth number in four species of the *Drosophila bipectinata* complex and their hybrids

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Summary. The number of teeth in the sex-combs of males of the *Drosophila bipectinata* complex differentiates species. In addition, the character distinguishes a majority of hybrids. A polygenic model of inheritance is proposed.

Key words. Drosophila bipectinata; sex comb, male; teeth number, sex comb; inheritance, polygenic; polygenic inheritance.

The *Drosophila bipectinata* complex includes four species (*D. bipectinata* (*bip*), *D. parabipectinata* (*par*), *D. malerkotliana* (*mal*) and *D. pseudoananassae* (*psn*). They occur in the Oriental-Australian biogeographic zones. All four species are sympatric in north-west Borneo and in Thailand[1]. The species are specially interesting for their morphological similarity coupled with their hybridizing ability. In the laboratory F_1 hybrids between all four species are obtained in no choice mating situations[1]. Natural hybridization has been reported between *bipectinata* and *malerkotliana* but it is rare[2]. Female F_1 hybrids are fertile and produce hybrids on backcrossing. Male F_1 hybrids are infertile.

The hybridizing ability of these species makes them prime subjects for genetic studies. On the other hand their morphological similarity makes laboratory procedures difficult.

The structure of the fore tarsal sex-comb in males is the only morphological character reported[1,3] which promises to be useful as a species and hybrid identifier. Color differences, namely, black abdominal terga in *malerkotliana* and *parabipectinata* contrasting pale tan in the other two species[1] cannot distinguish all species from each other. No other morphological difference has been reported. Females are indistinguishable and examination of male genitalia reveals no differences[1,3].

This paper describes variability in sex-comb teeth number within species and species hybrids of the *D. bipectinata* complex. The aim is to assess the suitability of this character for distinguishing genetically distinct populations. Differences in male abdominal tergite coloration and teeth location are also assessed. The results are relevant to a study of the genetic architecture of courtship song in this complex which is in progress in our laboratory and will be reported elsewhere. A second aim is to formulate hypotheses concerning the inheritance of sex-comb teeth number.

The experimental procedure was to randomly select 20 male flies from each of the four species. The species had been maintained in our laboratory for seven years and were presumably fairly inbred. Laboratory culture does not necessarily imply inbreeding but in this case stocks had been reduced markedly on several occasions. Hybrid males were obtained from vials in which five virgin males of one species were housed with five virgin females of another species. There were six possible crosses between the species and six reciprocal crosses making a total of twelve. Data were collected from 20 males of each kind of hybrid.

Teeth are arranged singly or in rows to form sex-combs on the tarsi of the front legs. *D. malerkotliana* and some of its hybrids have teeth on the 3rd tarsal segment as well as on the metatarsus (1st tarsal segment) and the 2nd tarsal segment. Teeth in the remaining species are confined to the first two tarsal segments. The total number of teeth varies within and between species and hybrids. In this study, all measurements are of the right fore tarsus.

Table 1 gives the mean number of teeth and the standard errors for each species. Differences between species were assessed with the Tukey test[4] following a preliminary one-way analysis of variance ($F_{3,76} = 139.32$, $p < 0.001$).

All species were significantly different from each other in mean teeth number ($p < 0.01$ except for *malerkotliana* and *pseudoananassae* where $p < 0.05$). The conclusion is that sex-comb teeth number is a character of use in distinguishing the four species.

Hybrid data are presented in table 2. Differences between hybrids and parents and hybrids and the mid parental value were calculated with t-tests. (In three comparisons concerning *pseudoananassae* variances were unequal. They were stabilized by subjecting the data to reciprocal transformation.)

Hybrids between *bipectinata* and *parabipectinata* and *bipectinata* and *malerkotliana* had mean teeth numbers which differed significantly from both parental values but which did not differ from the mid parental value. Reciprocal hybrids did not differ significantly from each other in either kind of cross. Sex-comb teeth number is useful, therefore, in distinguishing hybrids of these two crosses from their parental species.

In the cross *parabipectinata* by *malerkotliana* the hybrids do not differ from the mid parental value and reciprocal hybrids do not differ in sex-comb teeth number. Hybrids can be

Table 1. Mean teeth number in the fore-tarsal sex combs of species in the *D. bipectinata* complex. N = 20. ( ) denotes SE

Species	$\bar{X}$ sex-comb teeth number
D. bipectinata	16.70 (0.22)
D. parabipectinata	12.90 (0.34)
D. malerkotliana	10.75 (0.27)
D. pseudoananassae	9.75 (0.19)

Table 2. Mean teeth number in the fore tarsal sex-combs of hybrid males. N = 20. ( ) denotes SE ($p < 0.05$*, $p < 0.01$**, $p < 0.001$***)

Male Female	Midparent	t_1	t_2	t_3	t_4
bip × par 15.05 (0.21)	14.80	0.26	5.43***	5.38***	1.14
par × bip 14.60 (0.34)	14.80	0.13	3.56**	5.24***	
bip × mal 12.15 (0.34)	13.73	1.01	11.20***	3.21**	1.38
mal × bip 12.75 (0.26)	13.73	0.82	5.27**	11.46***	
par × mal 12.20 (0.30)	11.83	0.27	1.53	3.56**	1.77
mal × par 11.40 (0.34)	11.83	0.28	1.51	3.14**	
psn × bip 12.30 (0.50)	13.25	0.40	4.87**	7.79***	3.98***
bip × psn 14.70 (0.33)	13.25	0.96	5.02***	12.90***	
psn × para 10.70 (0.30)	11.23	0.39	2.68*	4.86***	6.35***
par × psn 13.60 (0.34)	11.23	1.50	1.45	11.15***	
psn × mal 11.30 (0.25)	10.25	0.91	4.90***	1.49	1.08
mal × psn 10.90 (0.27)	10.25	0.52	0.39	3.48**	

t_1, difference between hybrid and midparent; t_2, difference between hybrid and maternal species; t_3, difference between hybrid and paternal species; t_4, difference between reciprocal hybrids.

distinguished from their paternal but not their maternal species by their sex-comb teeth number. Hybrids between *pseudoananassae* and *bipectinata*, and *pseudoananassae* and *parabipectinata* are distinguishable from their paternal species in sex-comb teeth number and from their maternal species except when the female parent is *parabipectinata* (i.e. *par* female × *psn* male). Reciprocal hybrids for these two crosses are also distinguishable and this is unlike any other reciprocal hybrids formed within the complex.

In the remaining cross, *pseudoananassae* by *malerkotliana*, the only significant difference was between the sex-comb teeth number of the hybrid and that for *pseudoananassae* irrespective of whether this was the paternal or the maternal species. Hybrids in this cross are therefore distinguishable from *pseudoananassae* but not from *malerkotliana*.

Male abdominal tergite coloration is reported for dead mature flies. Variability of living forms obscures group differences. The general body color is tan. Tergites 5 and 6 are shiny black in *parabipectinata* and dull black in *malerkotliana*. Tergites 3 and 4 are also dark, more extensively so in *malerkotliana* than in *parabipectinata*. Markings on the abdominal tergites of *bipectinata* and *pseudoananassae* consist of a brown band along the posterior border of each tergite. Bands of color are wider in *pseudoananassae* giving it a darker appearance than *bipectinata*.

Hybrids were darker than their lightest parent and lighter than their darkest parent. In most crosses reciprocal hybrids were different being closest in color to their maternal species. Reciprocal hybrids which were least different were those between *bipectinata* and *parabipectinata*, dull brown, and *malerkotliana* and *parabipectinata*, shiny black to dull black. Hybrids between *pseudoananassae* females and *bipectinata* males were *pseudoananassae* like. Color in the reciprocal hybrid, which occurs infrequently, was not recorded.

The presence of teeth on the 3rd tarsal segment is a reliable character for distinguishing *malerkotliana* from the other species in the complex. Of the different kinds of *malerkotliana* hybrids, only two (*mal* female × *bip* male and *par* female × *mal* male) lacked this character. Some of each of the other kinds of hybrid possessed it.

In conclusion, sex-comb teeth number distinguishes the species under study. It also distinguishes eight of the twelve kinds of hybrids from their maternal species and eleven from their paternal species. The twelth hybrid (*psn* female × *mal* male) can be identified because its color is like the female parent but it has 3rd tarsal segment teeth like the male parent. The latter character and color are not useful in distinguishing the four remaining hybrids from their maternal species.

The pattern of inheritance of sex-comb teeth suggests a polygenic model with genes located on the autosomes. The evidence is that no kind of hybrid differed from the mid parental value. In two kinds of crosses (*psn* × *bip* and *psn* × *par*) reciprocal hybrids were different. Therefore these three species (*psn, bip* and *par*) may also carry genes for sex-comb teeth number on the X-chromosome. A study of backcrosses between female hybrids and their paternal species is in progress to test genetic hypotheses.

The functional significance of teeth number in sex-combs is unknown. Elimination of tapping by removing fore tarsi decreases the sexual isolation between *D. malerkotliana* and *D. bipectinata*[5]. Perhaps the sex combs are scent receptors and teeth number reflects evolutionary divergence in responsiveness to species specific scents.

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Spontaneous aneuploidy of chromosome 4 in *Drosophila kikkawai* in Thailand[1]

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Summary. In *D. kikkawai* (2n = 8), chromosome 4 aneuploidies up to heptasomy, possibly connected with spontaneous nondisjunction, translocation and centric dissociation, have been observed in one isofemale line collected from Mae Hong Son, northwest Thailand. This wide range of numerical and structural variation persisted in the laboratory for many generations.
Key words. *Drosophila kikkawai;* chromosome 4; aneuploidy.

The eukaryotic genome is usually invariable in terms of chromosome number. The gain or loss of heterochromatic material in chromosome complements may occur in nature without causing severe phenotypic or genetic effects[2,3]. Conversely, the increase of euchromatin in the genome, even a small portion, might be expected to produce marked genetic effects. Yet chromosomal nondisjunction producing aneuploidy is not an uncommon phenomenon in higher organisms[4,5].

We report here some interesting cases of spontaneous aneuploidy of chromosome 4 in *D. kikkawai* from Thailand. In earlier studies of this species no such chromosomal variation was noted[6].

Materials and methods. F_1 larvae were cytologically examined from individual ovipositions of 42 females of *D. kikkawai* caught in Mae Hong Son, northwest Thailand.The larval mitotic chromosomes were prepared from brain ganglia using an air-dried Giemsa staining technique[7]. Three isofemale lines showed variation in chromosome number with respect to microchromosome 4. One of the three families (A76-7) has been maintained in the laboratory as mass cultures as well as pair matings to follow the aneuploid condition in the culture stock until the 21st generation. Photomicrographs of metaphase chromosomes were made with Kodak High Technical film under oil immersion (×670) with a green filter.

Results and discussion. The normal metaphase karyotype of *D. kikkawai* is 2n = 8. The 4th chromosome (microchromosome) varies in size and shape depending on the amount and distribution of major heterochromatic blocks, ranging from a small telocentric to a large metacentric configuration[6]. Natural populations of *D. kikkawai* in the Oriental region, including Thailand, generally exhibit three types of 4th chromosome; a large telocentric (Lt), a medium metacentric (Mm) and a large submetacentric chromosome (Lsm) (fig. 9). Of the 42 isofemale lines observed in this study three, including No. A76-7, showed